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Metastasis to the lung is a common occurrence accounting for approximately 60-70% of metastasis. We have developed a transgenic model of lung metastasis using a MMTV-c-myc/MMTV-VEGF bitransgenic mouse system. We are using this system to evaluate the mechanism by which VEGF is able to dramatically increase metastasis to the lung. Solid progress has been made during a no-cost extension year granted to allow us to re-group and overcome technical challenges that have slowed the anticipated accrual of tumor material for the study. Three publications are in the final stages of publication which have been directly supported by this award.

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Introduction:

Angiogenesis is a process that has been shown to be of critical importance to mammary tumor development growth and dissemination. There is a dearth of models for the study of these tightly linked processes – angiogenesis and metastasis, since none of the available human breast cancer cell lines are metastatic in the nude mouse and many of the available mouse models are driven by oncogenes not normally overexpressed in human tumors.

The oncogene c-myc is highly relevant to human cancer, however, being amplified or overexpressed in a significant proportion of human breast tumors. Mice that carry a transgene in which the MMTV (Mouse Mammary Tumor Virus) promoter directs the expression of c-myc to the mammary glands, develop mammary tumors after multiple pregnancies. However, these tumors are relatively poorly vascularized and typically only form rare, small metastases in the lungs of the mice.

We hypothesized that increasing the expression of the angiogenic growth factor VEGF (Vascular Endothelial Growth Factor) in these tumors would result in increased vascularization of the tumors and increased metastatic potential. In order to achieve this goal, we crossed the MMTV-c-myc mice with another transgenic mouse strain which carry a MMTV driven VEGF expression cassette. The frequency and latency of tumor formation in these mice is unchanged, but as hypothesized, the tumors are much more vascular and metastasize more frequently forming larger secondary tumors.

VEGF-A is highly relevant to human tumors. Many human breast cancers express significant levels of VEGF and so the model we have created accurately mimics at least one variety of invasive and metastatic mammary tumor. It is reassuring that by standard histological criteria the tumors that occur in these mice indeed look very similar to human breast cancers and are quite distinct from the tumors that naturally occur spontaneously within the mammary glands of mice, or those that are seen in carcinogen induced models of mammary carcinogenesis.

Body:

Progress on research:

As indicated in the last annual report, we have somewhat been dogged by technical challenges. It is in the nature of Idea grants that unexpected pitfalls occur and need to be overcome. Last year we indicated that we would be requesting a no-cost extension for the project to provide us with time to re-group, re-grow the c-myc-VEGF colony and so develop the necessary materials to complete the work described. The no-cost extension was granted and have made use of the time that this has given us to continue to expand the colony and allow tumors to form.

We have discovered that the MMTV-c-myc transgene is very susceptible to subtle alterations in the genetic background of the mice that carry it in terms of the penetrance

of the tumor phenotype and the latency to tumor formation. We have recently completed a paper describing this phenomenon which has been submitted to the journal Transgenic Research for publication. The manuscript received favorable review and we are in the process of addressing the comments raised and hope that it will be accepted for publication shortly. The findings described in this manuscript are threefold. 1) that this background effect can significantly reduce the penetrance of transgenes – including c-myc, even when the mixed background is very similar to the starting background – as it is in the case of the c-myc-VEGF cross – both are on a FVB background – though from different sources. 2) that a similar effect can be seen on the latency to tumor formation in the animals that do get tumors, and 3) that these effects can be significantly amplified or to some extent mitigated by alterations in the age of the mice when they first become pregnant.

Other than allowing us to prepare an interesting manuscript (which gratefully acknowledges this award for support), the effects we describe have caused us to continue to have a difficult time accruing the tumors that we need to complete the histochemistry studies. Nevertheless we have made significant progress and believe that we will be able to finish the work in the year remaining for the award.

Work with the c-myc and c-myc/VEGF lines has progressed. We have now completed a thorough characterization of the *in vitro* and *in vivo* properties of these cells and have proceeded with transfection with enzymatic and fluorescent tags to allow completion of the intravasation and extravasation studies to be conduced in the final year of the project. In the last annual report we mentioned that a paper describing the methods used to isolate the cell lines to be used in this study and the initial characterization of those cell lines had been submitted for publication. That paper received favorable review; however the reviewers requested some additional experiments. We have been working on the final figure for the paper over the last few months and will re-submit the paper in what we hope will be final form in the next few days. Similarly, the paper by Nobel et al described as in preparation in the previous report has not progressed as fast as we would like and has yet to be submitted. The histology of some of the tumors described in this work was rather confusing and so at the recommendation of the pathologist on the study, several of the studies described therein have been repeated. We anticipate submission of this paper in approximately 1 month.

Key Research Accomplishments:

1) We have continued accruing mice and tumors to the study
2) We have established the staining methods to be used in the study
3) We have transfected the c-myc and c-myc/VEGF lines for use in the study with enzymatic and fluorescent marker proteins.

Reportable Outcomes:

Early Parity Significantly Elevates Mammary Tumor Incidence in MMTV-c-myc Transgenic Mice M. Hunter Jamerson1, Michael D. Johnson1, Priscilla A. Furth1 & Robert B. Dickson – submitted.

VEGEF-dependent mammary tumor metastasis in a novel bitransgenic model. Nobel et al, in preparation.

Explant-cell culture of primary mammary tumors from MMTV- c-myc and MMTV-c-myc/MMTV-VEGF transgenic mice. Pei et al, in preparation.

Conclusions:

We have made good use of the no-cost extension granted by the DOD for this project to re-group and build the resources we will need to complete the study as laid out in the SOW. We have finalized 3 papers which we believe will be accepted for publication in the next few weeks all of which were supported by this award, and which describe work that will greatly advance our understanding of breast cancer and in particular the utility of specialized animal models in its study.

Abbreviations:

βgal	Betagalactosidase
GFP	Green Fluorescent Protein
IHC	Immunohistochemistry
MMTV	Mouse Mammary Tumor Virus
RFP	Red Fluorescent Protein
VEGF	Vascular Endothelial Growth Factor